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THE EFFECTS OF FOOD DEPRIVATION AND SALINITY CHANGES ON REPRODUCTIVE FUNCTION IN THE ESTUARINE GOBIID FISH, *GILLICHTHYS MIRABILIS*

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Photoperiod and temperature are frequently considered the most important proximate factors regulating teleost reproductive cycles (de Vlaming, 1972); yet few investigators have examined the influence of other environmental factors, such as changes in food availability, salinity, and oxygen concentration. A previous investigation (de Vlaming, 1971) showed that the spawning period in the estuarine gobiid fish, *Gillichthys mirabilis*, is protracted, extending from December to June. Gonadal regression occurs rather abruptly in July; the gonads remain regressed during August and September. Gonadal recrudescence begins in late September, reaching completion by early December. Evidence presented by de Vlaming (in preparation) indicates that the increasing temperatures of summer may be responsible for terminating reproduction in the Alviso population of *G. mirabilis*. Carpelan (1957), however, in a study of the hydrobiology of the Alviso ponds, showed that there is a decrease in productivity, increase in salinity, and decrease in oxygen concentration in this habitat during the summer.

While there is a general awareness of a nutritional influence on fertility and fecundity, little information is available concerning specific nutritional effects on the gonads of fishes (Fontaine and Fontaine, 1962). Bagenal (1967), Nikolsky (1963), and Woodhead (1960) reviewed the literature on fish fecundity, and indicated that fecundity generally decreases with decreasing food availability. With regard to salinity, Kinne (1964) stated that this factor usually affects reproduction of marine and brackish animals less obviously than temperature. Kinne also suggested that salinity changes are seldom of importance in timing annual breeding cycles. The effects of salinity on fish reproduction, however, have been investigated in only a few species; nonetheless, in the Baltic Sea sterility or reduced reproductive potential due to low salinity has been reported for several pleuronectid fishes (Marx and Henschel, 1939).

The seasonal timing of gonadal regression in *G. mirabilis* suggests that decreasing food availability or increasing salinity could be implicated. Accordingly experiments were designed to assess these possibilities.

Materials and Methods

To determine seasonal variation in fattening, monthly samples of *Gillichthys* from the Alviso ponds of San Francisco Bay (37° 27′ N) in California were obtained by trapping with modified minnow traps. Samples were usually taken near the middle of each month. Collections were begun in December 1969 and con-

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tinued until October 1970. Formalin (8%) preserved specimens of *Gillichthys* were obtained from the Scammons Lagoon population (27° 48′ N) in Baja California between April and October 1970. The fish from the Alviso population were killed on the day of collection using a saturated solution of chlorotone. Only males longer than 125 mm and females longer than 120 mm were used in these studies to keep animal size as uniform as possible.

Samples of fish for experimental purposes were captured in the Alviso habitat at different times during the year and thus in different phases of gametonenesis. Several fish from each sample were sacrificed and the gonads examined at the time of capture; these fish, the initial controls, served as a reference for the experiments that followed.

Gonadal weights are expressed in absolute terms since it has been shown (de Vlaming, 1971) that gonadal weight is independent of body weight (and length) in the size of fish utilized. Gonads were fixed in Bouin's, Zenker's, alcoholic Bouin's, or Zenker-formol solutions and embedded in paraffin for histological examination. Tissues were sectioned at 5 μ–8 μ and stained with Delafield's, Harris', or Heidenhain's haematoxylin and counterstained with eosin or light green. Some

TABLE I

Criteria used in evaluating gametogenetic activity in G. mirabilis

Stage	Histological characteristics of testes
0	"Regressing testis." Seminiferous lobules characterized by large numbers of pyknotic nests of degenerating cells (spermatozoa, spermatids, and spermatocytes); phagocytes observed free within the lobules.
1	"Quiescent testis." Seminiferous lobules small in diameter. Germinal epithelium consists of only few residual spermatozoa, and the sperm duct is collapsed.
2	"Mitotic phase." Same as Stage 1, with the exception that mitotic figures are observed in the spermatogonia.
3	"Meiotic phase or active spermatogenesis." Testicular lobules larger than in Stages 1 and 2; germinal epithelium consists of spermatogonia, spermatocytes, and spermatids.
4	"Pre-spawning testis." Seminiferous lobules large and distended with sperm. Germinal epithelium consists of relatively few spermatogonia.
5	"Post-spawning testis." Seminiferous lobules small and contain relatively few sperm; sperm duct expanded and containing residual sperm.
	Histological characteristics of ovaries
I	"Regressing ovary." Atretic follicles predominate in the ovary. Only non-yolky oocytes and oogonia present.
II	"Quiescent phase or phase of oogonial proliferation." Ovary characterized by non-yolky oocytes with a basophilic cytoplasm, and a diameter of less than 75 μ. Granulosa not fully organized around the developing oocytes.
III	"Phase of active vitellogenesis." Ovary characterized by developing yolky oocytes whose diameter is between 75 μ and 640 μ. Granulosa fully organized around the oocytes.
IV	"Pre-spawning condition." Ovary characterized by oocytes whose diameter is in excess of 640 μ. Yolk vesicles abundant.
V	"Post-spawning condition." The ovary is wine-red in color; the tunica albuginea thick, highly vascularized, and folded. Post-ovulatory follicles predominate in the ovary. The stroma of the ovary appears disorganized, yet highly vascularized.

testes were also stained with Sudan Black B or by the PAS technique (Humason, 1962). Spermatogenesis and oogenesis were divided into six and five recognizable phases (Table I), respectively, to facilitate quantitative evaluation of gametogenetic activity. These phases have been previously described by de Vlaming (1971).

Stage III of this arbitrary classification of ovaries could be divided into several phases of vitellogenesis; however, a single category is used here to denote a stage of active vitellogenesis. Egg diameter is not used since oocyte development is not synchronous in this species (de Vlaming, 1971); oocytes of varying diameter, and different phases of vitellogenesis characterize ovaries undergoing active gametogenesis.

In salinity experiments, increased osmotic concentrations were achieved by adding Seven Seas Marine Mix (Utility Chemical Co.) to natural sea water; osmotic concentrations were determined by using an American Optical refractometer (Model 10402). Concentrations were maintained at a constant level in each experiment. The fish in these experiments were provided with a varied diet consisting of brine shrimp, chopped fish, boiled egg-white, beef kidney and liver. In the starvation experiments, the weight-length ratio of each fish was determined by dividing body weight (less the weight of the gonads) by the standard length. The hepatosomic index was determined by dividing the liver weight by the body weight (less the weight of the gonads). These two indices reflect the robustness (and hopefully nutritional state or energy reserves) of the fish.

Experimental fish were maintained in 56- or 132-liter tanks. Recirculating filtered water was used in all of these experiments. Tanks were housed in constant temperature rooms (± 1.5° C) and water pH maintained between 8.0 and 9.5 (which is consistent with the Alviso habitat).

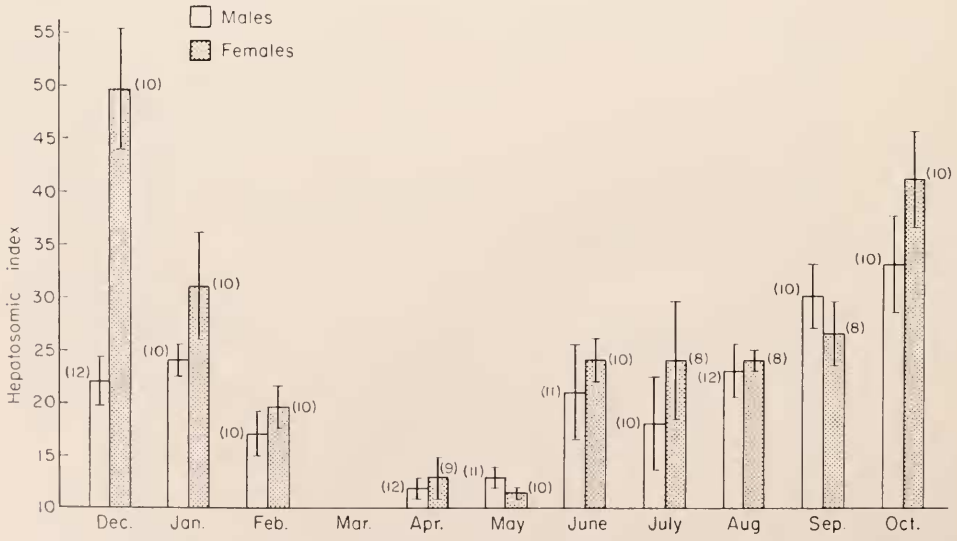

FIGURE 1. Seasonal variation (Dec. 1969–Oct. 1970) in hepatosomic index of *G. mirabilis* from Alviso population. Histograms (shaded = females; open = males) represent means; means are bracketed by one standard error. Sample size is shown in parentheses; hepatosomic index = (Liver wt/Body wt less gonadal wt), × 100.

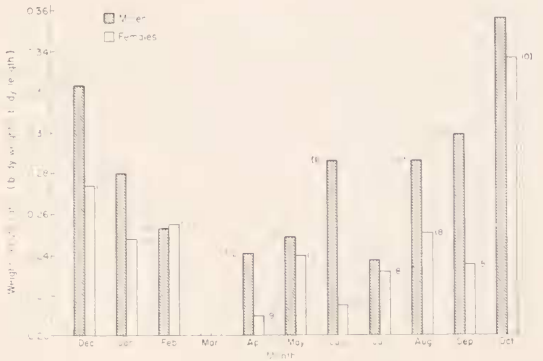

FIGURE 2. Seasonal variation (Dec. 1969–Oct. 1970) in weight-length ratio of *G. mirabilis* from Alviso population. Histograms (shaded = males; open = females) represent means; standard error less than 0.005 in all groups. Sample size is shown in parentheses; weight-length ratio = Body wt (g) less gonadal wt/Body length (mm).

Statistical comparisons of gonadal weights between experimental groups were made by using the Mann-Whitney U test (Siegel, 1956). This nonparametric test is suitable for small sample sizes and can be used to determine whether two independent groups have been drawn from the same population.

Individual experiments were conducted to determine the effects of inanition on fish in a phase of active gametogenesis, on the rate of testicular regression at a high temperature and on gonadal recrudescence. Other experiments were initiated to examine the influence of high salinity on gonadal regression and recrudescence in *Gillichthys*.

RESULTS

Nutrition

Seasonal variation in hepatosomatic index and weight-length ratio. Seasonal changes in weight-length ratios and hepatosomic indices are illustrated in Figures 1, 2, 3 and 4. In the Alviso and Scammons Lagoon populations both the weight-length ratio and hepatosomic index begin to increase towards the end of the spawn-

TABLE 11

Effect of 3-week starvation on gonadal weight in G. mirabilis *(16°C)*

Treatment	Gonadal weight ($\bar{x} \pm$ S.E.)		Hepatosomic index ($\bar{x} \pm$ S.E.)	
	Testes (n) (mg)	Ovaries (n) (mg)	Male	Female
Initial controls (from natural population)	79.1 ± 9.5 (10)	474 ± 23 (8)	16.9 ± 2.3	18.6 ± 2.8
Fed	85.4 ± 6.2 (10)	506 ± 17 (8)	28.4 ± 5.0*	30.0 ± 3.5*
Starved	43.5 ± 4.5* (10)	245 ± 9* (8)	9.8 ± 1.3*	10.4 ± 0.9*

* Significantly different ($P < 0.01$) from initial controls.

ing season (May or June). These indices continued to increase as gonadal recrudescence was occurring (until October in the Alviso population and August in the Scammons Lagoon population). With the onset of spawning (December in the Alviso population and September in the Scammons Lagoon population) both the weight-length ratio and hepatosomic index began to decrease, and continued to decrease through the spawning season in the Alviso population. These data indicate that there is a seasonal variation in the robustness (and perhaps energy reserves) of *Gillichthys* which is correlated with the reproductive cycle.

The effects of inanition in January. To determine whether food shortage could initiate gonadal regression an experiment was begun in January when the testes of fish were in active spermatogenesis, the pre-spawning, or post-spawning condition (Stages 3, 4, and 5); the ovaries of fish in this sample were in phases of active vitellogenesis (Stage III). One group of fish was fed every other day, whereas another group received no food; both groups were placed at 16° C and sacrificed after 23 days (Table II). In healthy fish, gonads remain active at this temperature (de Vlaming, in preparation).

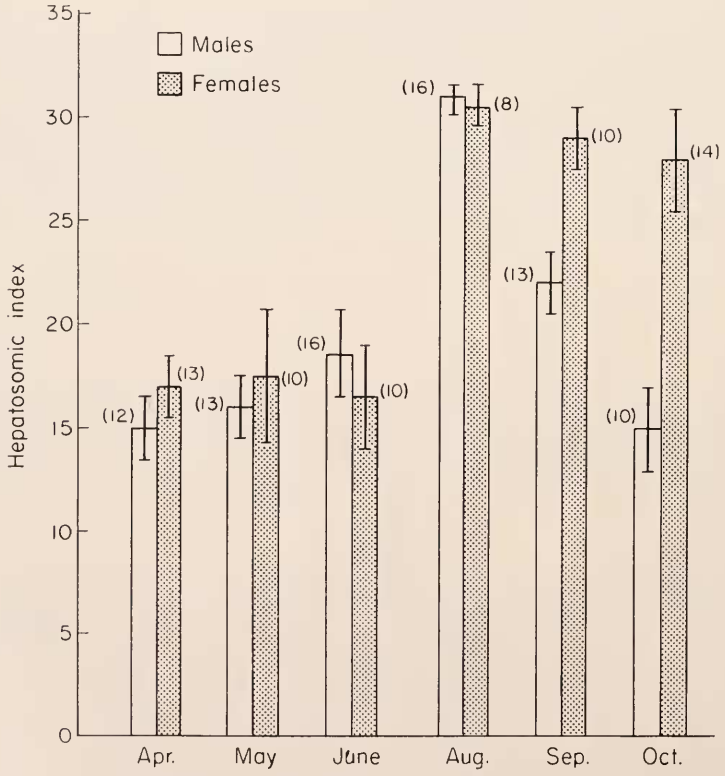

FIGURE 3. Seasonal variation (April–Oct. 1970) in hepatosomic index of *G. mirabilis* from Scammons Lagoon population. Histograms (shaded = females; open = males) represent means; means are bracketed by one standard error. Sample size is shown in parentheses; hepatosomic index = (Liver wt/Body wt less gonadal wt), × 100.

TABLE III

Rate of testicular regression in starved and fed G. mirabilis *at 27° C*

	Testicular weight (mg) $\bar{x} \pm$ S.E. (n)	Hepatosomic index ($\bar{x} \pm$ S.E.)	Body wt/body length** ($\bar{x}$)
Initial controls (from natural population)	89.2 ± 7.1 (10)	13.33 ± 1.04	0.259
Fed	67.8 ± 6.7* (10)	18.88 ± 2.13*	0.270*
Starved	55.3 ± 7.5* (9)	6.26 ± 0.88*	0.245*

* Significantly different ($P < 0.01$) from the initial controls.
** Standard error < 0.001 in all groups.

Inanition for 23 days caused significant decreases ($P < 0.01$) in both ovarian and testicular weights compared to the initial controls and fed fish. The testes of the starved fish were regressing or in the quiescent phase (Stage 0 or 1); ovaries of fish in this group were also regressing (Stage I). In contrast, the gonads of the fed fish remained in the initial condition. The significant decrease ($P < 0.01$) in the hepatosomic index of the starved fish indicates that energy reserves were taxed by the lack of food.

Rate of testicular regression in starved and fed fish at 27° C. A second experiment was undertaken in May to ascertain the effects of nutrition on the rate of heat-induced testicular regression; testes of the initial controls collected from the natural population were in active spermatogenesis or the pre-spawning condition (Stages 3 or 4). One group of fish was fed every day *ad libitum*, whereas another group received no food; both groups were placed at 27° C (which causes gonadal regression in fed fish, de Vlaming, in preparation) and sacrificed after ten days (Table III).

Testicular weights of fish in both the starved and fed groups were significantly lower ($P < 0.01$) than those of the initial controls, but were not significantly different from one another; the testes of all fish were regressing (Stage 0). The hepatosomic index and the weight-length ratio of the starved fish were significantly lower ($P < 0.01$) than those of the initial controls, but those of the fed fish had increased significantly ($P < 0.05$). Thus, in this short-term experiment the rate of testicular regression was not accelerated by starvation.

TABLE IV

Effect of 40-day starvation on gonadal recrudescence in G. mirabilis *(20° C)*

Treatment	Gonadal weight ($\bar{x} \pm$ S.E.)		Body wt/body length** ($\bar{x}$)	
	Testes (n) (mg)	Ovaries (n) (mg)	Male	Female
Initial controls (from natural population)	27.3 ± 1.3 (8)	114 ± 42 (8)	0.294	0.247
Fed	58.0 ± 3.7* (6)	408 ± 48* (6)	0.296	0.251
Starved	43.6 ± 4.1* (6)	351 ± 32* (6)	0.176*	0.181*

* Significantly different ($P < 0.01$) from initial controls.
** Standard error < 0.001 in all groups.

Effect of 40-day starvation on gonadal recrudescence (20° C). To determine whether diet limitations could inhibit the initiation of gonadal recrudescence the effect of starvation was examined in July when the testes and ovaries of the initial controls were regressing (Stage 0 and I). Controls were fed *ad libitum* every other day. Fish were placed at 20° C and sacrificed after 40 days (Table IV).

Testicular and ovarian weights of both experimental groups increased significantly ($P < 0.01$); active spermatogenesis (Stage 3) and vitellogenesis (Stage III) had been initiated in the gonads of all fish. Although the initiation of recrudescence was not blocked by inanition, the rate of recrudescence may have been reduced since the testicular and ovarian weights in the fed group were significantly higher ($P < 0.05$) than those of the starved group. The weight-length ratio of

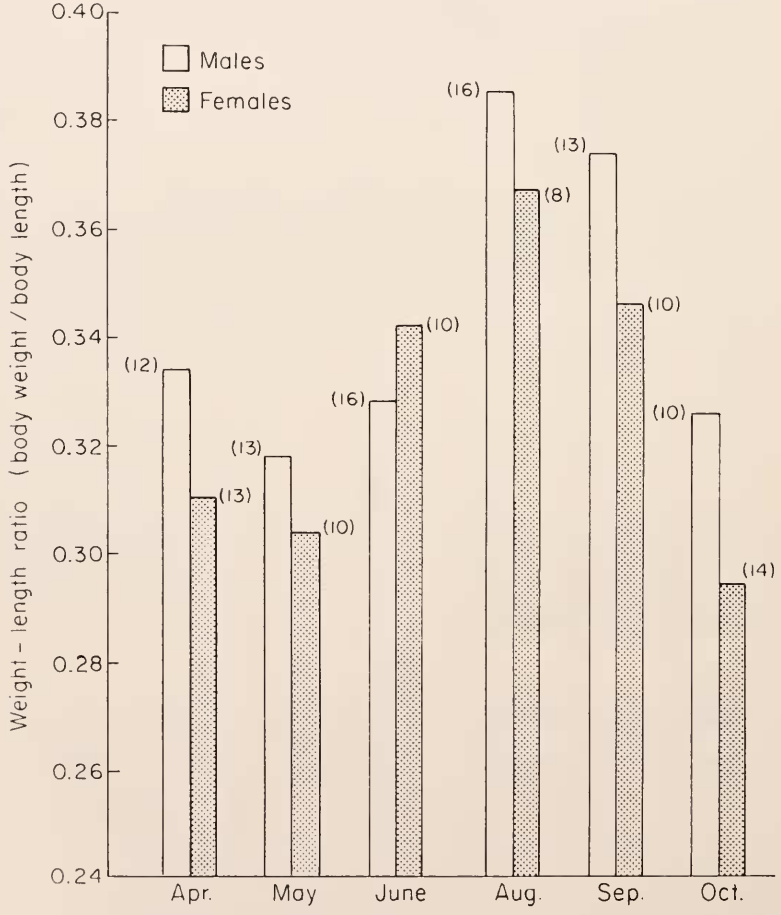

FIGURE 4. Season variation (April–Oct. 1970) in weight-length ratio of *G. mirabilis* from Scammons Lagoon population. Histograms (shaded = females; open = males) represent means; standard error less than 0.005 in all groups. Sample size is shown in parentheses; weight-length ratio = Body wt (g) less gonadal wt/Body length (mm).

TABLE V

Effect of 80-day starvation on gonadal weight in G. mirabilis *(16° C)*

Treatment	Gonadal weight ($\bar{x} \pm$ S.E.)		Hepatosomic index ($\bar{x} \pm$ S.E.)		Body wt/body length** ($\bar{x}$)	
	Testes (n) (mg)	Ovaries (n) (mg)	Male	Female	Male	Female
Initial controls (from natural population)	32.2 ± 2.2 (12)	125 ± 6 (8)	19.4 ± 7.7	26.9 ± 11.3	0.265	0.248
Fed	54.6 ± 4.1* (8)	342 ± 21* (8)	37.2 ± 6.4*	41.6 ± 5.8*	0.354*	0.329*
Starved	49.8 ± 3.9* (12)	309 ± 14* (8)	8.1 ± 1.1*	12.3 ± 0.5*	0.208*	0.184*

* Significantly different ($P < 0.01$) from initial controls.
** Standard error < 0.001 in all groups.

both male and female starved fish was significantly less ($P < 0.01$) than those of the initial controls, suggesting that starvation did cause a decrease in body weight.

Effect of 80-day starvation on gonadal recrudescence (16° C). The effects of longer-term starvation on gonadal recrudescence at a moderate temperature were again examined in July; the testes and ovaries of all of the initial controls were regressing (Stage 0 and I). The conditions employed were the same as in the previous experiment, except fish were placed at 16° C and sacrificed after 80 days (Table V).

Testicular and ovarian weights of both experimental groups increased significantly ($P < 0.01$); active spermatogenesis (Stage 3) and vitellogenesis (Stage III) were initiated in the gonads of all fish. Gonadal weights of the starved and fed fish were not significantly different. The hepatosomic index and weight-length ratio of both male and female starved fish were significantly less ($P < 0.01$) than those of the initial controls.

Salinity

Effects of high salinity on gonodal regression. To determine whether high salinity could induce gonadal regression, an experiment was begun in October in which the testes of the initial controls were in active spermatogenesis (Stage 3) and ovaries were in phases of vitellogenesis (Stage III). One group of fish was

TABLE VI

The effect of 20-day high salinity treatment on gonadal regression in G. mirabilis *(20° C)*

Treatment	Gonadal weight ($\bar{x} \pm$ S.E.)	
	Testes (n) (mg)	Ovaries (n) (mg)
Initial controls (from natural population)	68.4 ± 10.3 (10)	2394 ± 404 (10)
35 parts/thousand	90.1 ± 5.2* (9)	2458 ± 217 (6)
70 parts/thousand	75.7 ± 9.1 (10)	2263 ± 195 (5)

* Significantly greater ($P < 0.01$) than initial controls.

TABLE VII

The effect of 70-day high salinity treatment on gonadal recrudescence in G. mirabilis *(20° C)*

Treatment	Gonadal weight ($\bar{x} \pm$ S.E.)	
	Testes (n) (mg)	Ovaries (n) (mg)
Initial controls (from natural population)	23.9 ± 1.6 (8)	158 ± 24 (8)
35 parts/thousand	109.5 ± 8.7* (6)	1776 ± 152* (6)
70 parts/thousand	56.1 ± 3.6* (6)	453 ± 17* (5)

* Significantly greater ($P < 0.01$) than initial controls.

exposed for 20 days to a salinity of 35 parts per thousand (ppt) and another group to 70 ppt; both groups were at 20° C (Table VI).

Ovarian condition in both experimental groups remained at the initial level. Testicular weights of fish at the lower salinity increased significantly ($P < 0.01$), and were also significantly greater ($P < 0.05$) than those of fish at the high salinity. Histological examination, however, revealed that the testes of fish in both groups were in Stages 3 or 4. These data suggest that high salinity may reduce the rate of gametogenesis, but nonetheless, gonadal regression is not stimulated.

Effects of high salinity on gonadal recrudescence. To determine whether high salinity would prevent gonadal recrudescence in *Gillichthys* an experiment was begun in September 1967 in which the testes and ovaries of the initial controls were in the quiescent phase (Stage I and II). One group of fish was exposed for 70 days to a salinity of 35 ppt and another group to 75 ppt both at a temperature of 20° C (Table VII).

Testicular and ovarian weights in both groups increased significantly ($P < 0.01$), but those of fish in the low salinity group were significantly greater ($P < 0.01$) than testicular and ovarian weights in the high salinity group. The testes of all fish in the low salinity group were in the pre-spawning condition (Stage 4), whereas those of fish at 75 ppt were in Stage 3. The ovaries of all fish at the high salinity were in the early phases of vitellogenesis (Stage III), whereas those of the fish at 35 ppt were in later phases of vitellogenesis. These data indicate that high salinity does not block the initiation of gonadal recrudescence, but does reduce the rate of gametogenesis.

DISCUSSION

The effects of starvation on gametogenesis in *G. mirabilis* vary as a function of season. This variability in the response to inanition may depend on the energy reserves of the initial controls or susceptibility of the gonadotropin-producing system. The data presented here indicate that starvation can induce gonadal regression in a relatively short period in *Gillichthys* in phases of active gametogenesis. In sexually maturing *Salmo gairdneri* starvation also reduces the number of eggs brought to maturity by causing follicular atresia (Scott, 1962). Clemens and Reed (1967) also showed that spermatogenesis in *Carassius auratus* is terminated by diet limitations at any time of the year. An increase in feeding has been shown to hasten the onset of sexual maturity by a year in *Pleuronectes limanda* (Gross,

1949), *Clupea harengus* (Cushing and Burd, 1956) and *Salvelinus alpinus* (Rumnström, 1951), whereas poor feeding delayed maturity in *Perca fluviatilis* (McCay, Dilley and Crowell, 1928–9; Alm, 1954) and *Salmo trutta* (Bagenal, 1969).

Perhaps then, decreasing food availability and the increasing temperatures of summer act synergistically to cause gonadal regression in the Alviso population of *Gillichthys*. Temperature does indeed have a pronounced effect on fish when food availability is low. For example, Phillips, Livingston and Dumas (1960) showed that during starvation weight loss is increased in brook trout by approximately 10% for each 1° C rise in water temperature. High temperature alone, however, can cause gonadal regression in *Gillichthys* because in all of the experiments conducted by de Vlaming (in preparation) thermally regressed fish were well fed (weight-length ratios and hepatosomic indices did not decrease). In addition, data presented here imply that the rate of gonadal regression at high temperatures is not increased by starvation. Furthermore, the complete starvation used in these experiments probably represents a more severe nutritional stress than is actually encountered by fish in nature.

Although metabolic rate in fish increases with temperature, food consumption may not increase sufficiently to maintain fat reserves and body weight. Creach and Serfaty (1965) indicated that the gonads and muscles are the principle source of free amino acids for metabolism when *Cyprinus carpio* is subjected to starvation. In addition, Kinne (1960) reported that the efficiency of food conversion in *Cyprinodon macularis* is maximal at lower temperatures and salinities, declining at higher temperatures and salinities. Paloheimo and Dickie (1966), nonetheless, indicated that increases in temperature increase the rate of energy turnover, but do not otherwise alter the basic pattern of distribution and use of energy within the body of fishes. Moreover, the weight-length ratio and hepatosomic index in *Gillichthys* begins to increase as gonadal regression occurs so it seems unlikely that energy shortage causes gonadal regression. Mann (1965) has also reported that, in several species of fish, withholding food reduces metabolic rate 50% within seven days. Possibly, however, food restriction leads to gonadal regression indirectly by causing stress and subsequent changes in the endocrine system.

The data presented here indicate that starvation will not block the initiation of gonadal recrudescence in *Gillichthys*. In contrast, Wilkins (1967) noted that starved *Clupea harengus* failed to undergo gonadal recrudescence. Assenmacher, Tixier-Vidal and Astier (1965) reported that starvation failed to prevent gonadal recrudescence in ducks, but fasting did induce involution of developing gonads. Sluiter, van Oordt and Grasvelt (1950) also showed that the effect of inanition on spermatogenesis in a frog, *Rana temporaria*, depends on the time of year; when fat bodies are large starvation has little effect, but when they are small inanition inhibits spermatogenesis. In this study, however, experiments on recrudescence were begun in July when the length-weight ratio and hepatosomic index of fish were relatively low (lower than in January when starvation brought about gonadal regression). These two indices should be indicative of the nutritional state of fish since Woodhead (1960) showed that the main source of fat and protein used during gonadal maturation comes from the liver and muscles. The variation in response to starvation in *Gillichthys* could be due to seasonal shifts in metabolism; such shifts are common in fish (Wells, 1935; Wohlschlag and Juliano, 1959; Beamish, 1964;

Roberts, 1964). However, temperatures are higher in early autumn (when starvation failed to prevent the initiation of recrudescence) and metabolism should be higher, than in January (when starvation caused testicular regression). In addition, Barlow (1961) reported that oxygen consumption remains the same throughout the year in *Gillichthys* from the Alviso population (measured at the same temperature).

If the weight-length ratio and hepatosomic index can be taken as an indication of fattening (or energy accumulation), then this process occurs during the autumn concomitant with gonadal recrudescence. Likewise, as spawning begins, these two indices begin to decline and continue to decrease through the spawning season, suggesting a depletion of energy reserves. Healey (1971) has also noted that changes in body weight in *Gobius minutus* are closely correlated with reproductive cycling. The decline in hepatosomic index during the spawning season (when estrogens should be high) is surprising since Kobayski (1953), Egami (1955) and Oguro (1956) showed that estrogens increase liver weight in fish. Perhaps in this species the prolonged spawning season places a burden on energy reserves, and energy expenditure overrides the estrogen effects on the liver. Indeed, a decrease in energy reserves during the spawning season might be expected since sex hormones increase the rate of oxygen consumption in fish (Raffy and Fontaine, 1930; Stanley and Tescher, 1931; Mann, 1939; Hasler and Meyer, 1942). According to Wilkins (1967), *Clupea harengus* exhibit their lowest fat content after the spawning season, and Lofts, Pickford and Atz (1968) indicated that *Fundulus heteroclitus* with regressed gonads have large livers. In *Gillichthys*, growth is fastest in the hot summer months (Walker, 1961) when the gonads are regressed.

Although variation in food availability is apparently not the proximate factor regulating reproductive cycling in *Gillichthys*, it well could be the ultimate control factor. Low food availability would probably not favor the survival of fry, and indeed, Ivlev (1961) found that younger fish have a shorter survival time than older fish in starvation experiments.

Studies with other euryhaline species indicate that salinity can influence reproduction. *Mugil cephalus* and *M. capito* cannot reproduce in freshwater (but spend part of each year there), and oocytes remain in the previtellogenic stage (Abraham, Blanc and Yashouv, 1966; Abraham, Yashouv and Blanc, 1967). The gonadotropin content of the pituitary of these *Mugil* species held in freshwater is considerably lower than that of pituitaries from sea-water fish (Blanc and Abraham, 1968), and the area of the pituitary containing the gonadotrophic cells is reduced in size in fresh-water specimens (Blanc-Livni and Abraham, 1970). Low salinities normally experienced by *Pleuronectes flesus* also block vitellogenesis (Solemdal, 1967).

Salinity in the Alviso ponds reaches a maximum in summer (August and September) when the gonads of *Gillichthys* are regressed; during this time salinity seldom exceeds 55 ppt in the ponds in which this species occurs (Carpelan, 1957). The experiments reported here (using salinities of 70 and 75 ppt) indicate that high salinity is not responsible for gonadal regression, nor will it prevent the initiation of gonadal recrudescence. Weisel (1948) also stated that the spermatozoa of *Gillichthys* are active in salinities ranging from 17 to 200% sea water. In combination with high temperatures, high salinity may induce gonadal regression, but changes in salinity alone cannot be considered a proximate factor in regulating reproductive cycling.

High salinity could act as an ultimate control factor with regard to reproductive cycling by influencing larval survival. This is doubtful, however, since Blaxter (1969), in a review of the literature, indicated that the salinity tolerance of larvae and eggs of marine fish is surprisingly wide.

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Summary

1. Investigations were conducted to examine whether decreasing food availability or increasing salinity might be implicated in termination of the breeding season in the estuarine gobiid fish, *Gillichthys mirabilis*.

2. Seasonal variation in weight-length ratio and hepatosomic index were studied in two populations of this species. These two indices, taken as an indication of fattening (or energy accumulation), seem to be correlated with the reproductive cycle. Both indices begin to increase as the gonads regress, and continue increasing concomitant with recrudescence; they decline steadily through the spawning season.

3. The effects of starvation on gametogenesis in this fish vary with season. Starvation can induce gonadal regression in a relatively short period in fish in phases of active gametogenesis, but does not block the initiation of gonadal recrudescence.

4. The rate of gonadal regression at high temperatures is not accelerated by starvation, suggesting that temperature can act independently to cause gonadal involution.

5. High salinity does not cause gonadal regression nor prevent recrudescence in *Gillichthys*.

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